Data Summary and Review on the Acute Toxicity of AE C656948 (Fluopyram) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....} EPA MRID Number 47372329

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	353315
	OECD Data Point	{.....}
	EPA MRID	47372329
	EPA Guideline	850.1075; 72-1

Test material: **AE C656948** **Purity:** 94.7%
Common name: Fluopyram
Chemical name: IUPAC: N-{2-[3-chloro-5-(trifluoromethyl)pyridine-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name: Not reported
CAS No.: Not reported
Synonyms: 3000312231 (Development number)

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	080302	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Nieden, D. 2006. Acute Toxicity of AE C656948 (tech.) to Fish (*Lepomis macrochirus*) under Static Conditions. Unpublished study performed by Bayer CropScience AG (Development – Ecotoxicology, Laboratory for Aquatic Organisms), Monheim, Germany. Laboratory project number E280 3142-0; laboratory report number EBGMP052. Study sponsored by Bayer CropScience AG (Portfolio Management, Project Management/Project Planning). Study completed on October 9, 2008. Report amendment submitted February 1, 2008.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, Bluegill Sunfish (*Lepomis macrochirus*) were exposed to AE C656948 at nominal concentrations of 0 (negative and solvent controls) and 6.0 mg/L; time-weighted average (TWA) concentrations of <0.1029 and 5.17 mg a.i./L under static conditions. The 96-h LC_{50} was>5.17 mg a.i/L. The EC_{50} and NOAEC values, based on lack of mortality or sub-lethal effects, were >5.17 and 5.17 mg a.i/L, respectively. Based on the results of this study, AE C656948 would be classified as practically nontoxic to Bluegill Sunfish (*Lepomis macrochirus*) up to the concentration tested in this study, on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified {scientifically sound or unsound} and {does or does not} satisfy the guideline requirement for an acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): 1.4 ± 0.1 g (mean ± SD); 4.8 ± 0.3 (mean ± SD)
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >5.17 mg a.i./L 95% C.I.: N/A
NOAEC: 5.17 mg a.i./L Probit Slope: N/A
EC_{50}: >5.17 mg a.i./L
Endpoint(s) Affected: None

I. REPORTED MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The method used in this study followed the recommendations of:

- U.S. EPA FIFRA § 72-1/SEP-EPA-540-85-006 (1982/1985)
- OPPTS 850.1075 (Public Draft, 1996)
- Directive 92/69/EEC, C.1 (1992)
- OECD No. 203 (rev. 1992)

COMPLIANCE:

Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided.
This study was conducted in compliance with:

- current OECD Principles of Good Laboratory Practice (GLP)
- current Principles of Good Laboratory Practice according to Annex 1 of the German chemical law (ChemG), dated June 20, 2002 [except for the screening work for contaminants in the dilution water]

USEPA-FIFRA Good Laboratory Practice Standards (40 CFR Part 160) as well as the GLP standards of the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 11 Nousan No. 6283 from October 1, 1999) [with the exception that recognized differences exist between the GLP principles/standards of OECD and the GLP principles/standards of FIFRA and JMAFF]

A. REPORTED MATERIALS:

1. Test material AE C656948

Description: Beige powder

Lot No./Batch No. : 08528/0002

Purity: 94.7%

Stability of compound under test conditions: After 96 hours, measured concentrations were 86-92% of nominal, indicating stability under test conditions.

Storage conditions of test chemicals: Room temperature

Physicochemical properties of AE C656948.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	

Parameter	Values	Comments
Kow	Not reported	

2. Test organism:

Species: Bluegill Sunfish (*Lepomis macrochirus*)
Age at test initiation: Not reported
Weight at study initiation: 1.4 ± 0.1 g (mean ± SD)
Length at study initiation: 4.8 ± 0.3 (mean ± SD)
Source: Osage Catfisheries, Osage Beach, Missouri, U.S.A.

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The study author reported that previously conducted solubility tests identified values around 6.0 mg/L as the practical limit of solubility.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details
Acclimation	
Period:	14 days
Conditions: (same as test or not)	Same
Feeding:	Fed daily with commercial trout food (Brutfutter Ecostart 17, BioMar, Denmark)
Health: (any mortality observed)	• <3% mortality prior to test initiation • All unsuitable fish (e.g. injured, deformed, etc.) eliminated prior to test group assignment. • Prophylactic pre-treatment with Oxytetracyclin-Hydrochlorid (4g/100L water, 2*24h) and therapeutic treatment with NaCl (15g/L, 30 min).
Duration of the test	96 hours

Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static N/A N/A
Aeration, if any	• Slight aeration at 24, 48 and 72 hours to maintain oxygen saturation >60%. • At 24 hours, aeration overnight; at 48 hours, aeration about 2 hours; and at 72 hours, aeration about 4 hours
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass 32 x 36 x 38 (L x D x H) 40 L
Source of dilution water Quality:	Reconstituted water prepared by adding salt stock solutions to demineralized water.

Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 40-60 mg $CaCO_3$/L 6.2-7.3 62-99% oxygen saturation <2 mg/L <5 mg/L Not detected (<0.1 or <1 µg/L) Not detected (<0.01 or 0.05 µg/L) <0.01 mg/L 21.5-22.5°C N/A Day 0, 2 and 4 of exposure period
Number of replicates/groups: control: solvent control: treated ones:	 2 2 2
Number of organisms per replicate /groups: control: solvent control: treated ones:	 15 15 15
Biomass loading rate	0.53 g fish/L test medium
Test concentrations: nominal: Time-weighted average:	 0 (negative and solvent controls) and 6.0 mg test item/L <LOD (<0.1029, negative and solvent controls) and 5.17 mg a.i./L
Solvent (type, percentage, if used)	Dimethylformamide (100 µL/L)
Lighting	16:8 hour light/dark photoperiod
Feeding	Not fed 48 hours before and during study
Recovery of chemical Frequency of determination Level of quantization Level of detection	 0, 48 and 96 hours 5 µg/L 1.7 µg/L
Positive control {if used, indicate	

the chemical and concentrations}	N/A
Other parameters, if any	N/A

2. Observations:

Table 2: Observations

Parameter	Details
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects
Observation intervals	0, 4, 24, 48, 72 and 96 hours
Were raw data included?	Yes
Other observations, if any	N/A

II. REPORTED RESULTS:

A. REPORTED MORTALITY:

Cumulative mortality after 96 hours was 0% among all fish in the controls and treatment groups.

Table 3: Effect of AE C656948 on Mortality of Bluegill Sunfish (*Lepomis macrochirus*).

Treatment (mg a i./L) Time-Weighted Average (TWA) and (Nominal)	No. of fish at start of study	Observation period					
		24 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (<0.1029)	30	0	0	0	0	0	0
Solvent control (<0.1029)	30	0	0	0	0	0	0
5.17 (6.0)	30	0	0	0	0	0	0
NOAEC	6.0 mg/L						
LC_{50}	>6.0 mg/L						
Positive control, if used mortality: LC_{50}:	N/A						

B. REPORTED SUBLETHAL TOXICITY ENDPOINTS:

No sublethal effects were observed among all fish in the controls or treatment groups after 96 hours.

Table 4: Sub-lethal Effect of AE C656948 on Bluegill Sunfish (*Lepomis macrochirus*).

Treatment (mg a i./L) Time-Weighted Average (TWA) and (Nominal)	Observation period		
	24 Hours	72 Hours	96 Hours
	% affected	% affected	% affected
Control (<0.1029)	0	0	0
Solvent control (<0.1029)	0	0	0
5.17 (6.0)	0	0	0
NOAEC	6.0 mg/L		
LOAEC	>6.0 mg/L		
EC_{50}	>6.0 mg/L		
Positive control, if used % sublethal effect: EC_{50}:	N/A		

C. REPORTED STATISTICS:

The study author did not perform statistical analysis and based toxicity values on the nominal test concentrations.

III. REVIEWER'S EVALUATION

A. DEVIATIONS FROM GUIDELINES: The following deviations from OPPTS guideline 850.1075 were noted.

1) Residual chlorine measured (<0.01 mg/L) possibly exceeded the maximum recommended level (0.003 mg/L).
2) Test material residues were observed in the test vessels during the study, but there was no mention of sample centrifugation prior to analytical determination.

B. OTHER STUDY DEFICIENCIES: None.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} , EC_{50} and NOAEC values were visually determined based on the observational data. The reviewer calculated the time-weighted average (TWA) concentration using the mean-measured concentrations from days 0, 2, and 4 and it is reported in the Executive Summary and Results sections of this DER (See Appendix I).

LC_{50}: >5.17 mg a.i./L 95% C.I.: N/A
NOAEC: 5.17 mg a.i./L
Probit Slope: N/A 95% C.I.: N/A

D. ADDITIONAL REVIEWER COMMENTS:

TWA concentration was reviewer-calculated (refer to associated Excel worksheet in Appendix I) and is reported in the Executive Summary and Conclusions sections of the DER. TWA concentration was calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C_{TWA} is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,.. n)
t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =48 hours).

The study author noted that "there was a prophylactic treatment directly after arriving of the fish with Oxytetracyclin-Hydrochlorid (4 g/100 L water, 2*24), beginning on February 7, 2006 and finishing on February 9, 2006. There was a therapeutic treatment against ectoparasites with sodium chloride (15 g/L, 30 minutes) on April 28, 2006".

The study author reported that no residues of the test item were detected in samples from the treated water control at 0 hours. However, a tiny amount of the test item was observed at the bottom and surface of the test

vessels at 24 hours. Tiny amounts of the test item were also observed at the bottom of the test vessels at 48, 72 and 96 hours. There was no report of test sample centrifugation prior to analytical determination.

The in-life portion of the definitive toxicity test was conducted from May 8-12, 2006.

E. CONCLUSIONS:

This study is/is not scientifically sound and does/does not fulfill the requirements for an acute freshwater fish toxicity study. The 96-hour LC_{50} value was >5.17 mg a.i./L. Due to a lack of treatment-related mortality or sublethal effects, the NOAEC was 5.17 mg a.i./L. Based on the results of this study, AE C656948 would be classified as practically nontoxic to Bluegill Sunfish (*Lepomis macrochirus*) on an acute toxicity basis up to the limit of water solubility for Fluopyram, in accordance with the classification system of the U.S. EPA.

LC_{50}: >5.17 mg a.i./L 95% C.I.: N/A
NOAEC: 5.17 mg a.i./L Probit Slope: N/A
EC_{50}: >5.17 mg a.i./L
Endpoint(s) Affected: None

REFERENCES:

Brauhn, J.L. and Schoettger, R.A. "Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegill Sunfish." Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975.

ASTM Standard E 729-1988. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. Philadelphia, PA.

APPENDIX I: REVIEWER'S TIME-WEIGHTED AVERAGE (TWA) CALCULATIONS:

Nominal (mg/L)	Time (hr)	Measured Conc	TWA (mg ai/L)	Mean TWA
6.0 (Rep A)	0	5.29		
	48	4.91		
	96	4.88		
			5.00	**5.17**
6.0 (Rep B)	0	5.69		
	48	5.24		
	96	5.22		
			5.175	